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PROBLEMS IN WATER ANALYSIS FOR PESTICIDE RESIDUES*

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SUMMARY

Organic solvents, glassware, plastic ware, cellulose extraction thimbles, filter paper, and silica gels may contribute contaminants to water samples which may interfere with the subsequent gas chromatographic analysis of the samples for pesticides in the parts per billion range. Prior to their use, heat treatment of the glassware and the silica gels is recommended to eliminate contaminants contributed by these materials. Plastic ware and filter paper should not be included in the analytical procedure.

INTRODUCTION

The examination of waters for pesticides has included samples of potable waters, fresh water streams, lakes, rivers, the oceans, and even sewage outfall areas. Without doubt, there will be non-pesticide chemicals in some samples that will possess analytical characteristics similar to some pesticides when they are examined by electron capture gas chromatography. In addition, false data may be acquired from extraneous sources during the manipulation of the samples in the analytical laboratory which will not be eliminated by confirmatory techniques, such as thin-layer chromatography (TLC), unless certain precautionary measures are taken prior to the analysis of the samples.

This report reviews some of these problems and also brings to the reader's attention several areas of analytical interferences which, to our knowledge, have not been specifically mentioned heretofore in the literature. The experienced analyst may be aware of these problems. However, with the increased interest in environmental studies coupled with the required establishment of many new laboratories possibly staffed with personnel inexperienced in trace analysis techniques, this report may aid the analyst in avoiding some unforeseen problems in the analysis of waters for pesticides.

When large samples of water (five or more gallons) are extracted for analysis and the extract is concentrated to a small volume, the suspected pesticide(s) in the water may be confirmed by TLC and spray reagent techniques if the pesticide residue

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in the extract is in the microgram range. However, the trend toward the use of small grab samples (one gallon or less) and the desire to find and report residues in the part per trillion, or fraction thereof, range eliminates the possibility of using the spray technique for verification, because of the detectability limits of the stain reagent. Under the latter conditions, the area of the developed TLC plate containing the suspected pesticide is eluted with a suitable organic solvent and the concentrated extract therefrom is again subjected to gas chromatographic analysis. Extraneous interferences are magnified on the recorder chart unless (1) special precautions are taken with the organic solvents, the glassware, and other equipment used in the analytical procedure and (2) the thin-layer adsorbent is completely free from organic contaminants.

EXPERIMENTAL

Organic solvents

Organic solvents of "reagent grade" quality cannot be used for pesticide residue analysis in the nanogram–picogram range, because of contaminants in the solvent which will be magnified on the gas chromatograph recorder chart when the final concentrated extract is applied to the gas chromatograph. It is inexcusable to use such reagents in the analytical procedure since high-purity solvents are now commercially available. If necessary, reagent-grade solvents should be redistilled in an all-glass system; however, the redistilled reagent should be checked before use.

Glassware and other equipment used prior to TLC and GC analysis of the water sample

LAMAR *et al.*[1] recommended heating all glassware, except volumetric ware, overnight at 300° prior to use with water samples. The volumetric glassware was cleaned with a solution of sodium dichromate in concentrated sulfuric acid. They also warned against the use of rubber, cork, or plastic stoppers for water sample containers. The Federal Water Pollution Control Administration water analysis manual[2] recommended heating the glassware at 400° if the type and size of glassware permitted such drastic treatment. AMOS[3] reported variable results—some satisfactory, some poor—when glassware was soaked in acid or base solutions or detergent solutions.

Plastic tubing used in vacuum equipment to remove sections of the silica powder from TLC plates have contributed organic contaminants to the powder[3,4].

Soxhlet extraction thimbles (Whatman cellulose) contain substances which will produce pseudo-pesticide peaks on the gas chromatogram unless the thimbles are solvent-extracted prior to use[3,5].

The following additional precautionary measures are suggested based on studies conducted in our laboratory. The glass jars used for developing the TLC plates may not tolerate the stress of heat treatment; therefore, sodium dichromate–sulfuric acid solution should be applied to the interior walls of the jar, followed by rinsing with water, acetone, and hexane. Whatman filter paper sheets are commonly used as liners in the chromatographic tank to saturate the interior of the tank with the vapors of the developing solvent. This practice cannot be tolerated in water analysis confirmatory work, because the paper may contaminate the developing solvent with organic materials which will be transferred to the TLC silica gel plate and finally to the concentrated eluted extract. Although the separation of certain groups of chlori-

nated pesticides may not be as efficient without the use of the paper liner, the eluted fractions from the TLC plates will give satisfactory results on the gas chromatograph for confirmatory analyses.

The syringes used for gas chromatograph samplings must be scrupulously clean and may require copious sequential washes with alcohol, acetone, and hexane accomplished by passing the solvents through the barrel of the syringe with the aid of a vacuum pump or water aspirator apparatus.

The inclusion of any glassware which contains ground-glass sections, such as glass-stoppered centrifuge tubes or volumetric flasks, will add to the analytical problem. Heating the glassware will not remove the contaminants from the ground, glass sections. Lengthy periods of washing with copious amounts of solvents may clean the ground-glass areas, but the procedure is impractical. If this type of glassware must be used, the contents of the container should not be poured from one container to another; the transfer should be made preferably with clean, heat-treated, disposable glass pipettes.

Four cleaning procedures for glassware were examined, each of which consisted of the same initial preparation as follows: Silica gel (0.1 ml dry volume), known to contain organic contaminants, was added to each of a series of centrifuge tubes (Kontes No. 410550, 5 ml capacity) and also to each of a series of Chromaflex sample tubes (Kontes No. 422560, 2 ml capacity); only 0.01 ml dry volume of gel was added to each sample tube. Hexane (0.5 ml) was added to each tube; the contents were coated on the interior walls of the tube by means of agitation on a vortex mixer. The contents of each tube was discarded. With preknowledge of the organic contaminant content of the silica gel, the above-mentioned amounts of gel were added to each tube to approximate the amount of gel that would be scraped from a TLC plate for further study and which would also approximate the amount of background contamination observed on the gas chromatograph recorder chart. Each tube was then washed with tap water and a nylon-bristle brush to remove adhering particles of gel from the walls of the tubes. Each tube was then rinsed with copious amounts of distilled water. The four subsequent cleaning procedures with sets of the above-mentioned contaminated tubes are outlined in Table I. Auxiliary glassware used throughout the analytical procedure was cleaned in a similar manner.

Subsequent to the cleaning procedures outlined in Table I, 0.5 ml of redistilled

TABLE I

RINSING SOLUTIONS IN GLASSWARE CLEANING PROCEDURES

Method			
1	*2*	*3*	*4*
Ethanol[a]	Dichromate–H_2SO_4[b]	Acetone	Dichromate–H_2SO_4[b]
Acetone	Tap water		Tap water
Hexane	Distilled water		Distilled water
	Acetone		Acetone
Air-dry	Air-dry	Air-dry	Air-dry
		Heat[c]	Heat[c]

[a] Glassware rinsed three times with each solvent in order of the listed sequence.
[b] Glass was soaked for 16 h in a solution of sodium dichromate–concentrated sulfuric acid.
[c] Glass was heated in an air oven for 16 h at 200°.

hexane was added to each centrifuge tube by means of a heat-treated disposable pipette, to avoid contacting the ground glass area of the tube with the solvent. The tube was then agitated for about 30 sec on a vortex mixer to wash the wall of the tube with the solvent, again being careful not to wet the ground glass area of the tube. The hexane was transferred, by pipette, from the centrifuge tube to the Chromaflex sample tube. The procedure was repeated four times, combining all hexane fractions in the sample tube. The hexane content of the sample tube was concentrated to about 0.02 ml with the aid of a stream of filtered nitrogen, and aliquots of the solution were applied to the gas chromatograph to check the efficiency of the glass cleaning procedure.

Referring to Table I, method No. 1 will not completely remove contaminants from the glassware. Methods 2, 3, and 4 will remove all contaminants and any one of the three procedures can be recommended. However, because of its relative simplicity, method No. 3 is preferred. It is apparent that organic solvents alone will not remove firmly bonded organic contaminants from glass; the more drastic treatment with an oxidizing reagent and a concentrated mineral acid and/or heat are prerequisites for contaminant-free glass equipment.

SCHAFER *et al.*[6] studies on pesticides in "drinking" waters described a stirring bar mechanism to mix thoroughly hexane and the water sample in gallon jugs for the extraction of the pesticides. Using this technique, it has been our experience that if Teflon magnetic stirring bars are used, the water sample will be grossly contaminated if the Teflon bars have been in previous contact with plant extracts or other biological media. If one contemplates using this mixing technique in water analyses, only new Teflon bars should be used and they should be screened for possible contamination properties prior to use.

Silica gel adsorbents

The transition in the past 10–15 years from the milligram range to the nanogram–picogram range in chemical analysis techniques must be considered in the following discussion on silica gel adsorbents. MILLER AND KIRCHNER[7] noted that silicic acid adsorbents contained as much as 100 mg of a yellow oily material in 100 g of adsorbent which was soluble in ethyl acetate or acetone and which, if present, interfered with UV and fluorescein tests on the chromatograms. STANLEY *et al.*[8] prewashed silica gel TLC plates with petroleum ether, followed by a continuous wash for 4 to 16 h with ethyl alcohol, to remove organic materials that would interfere with diphenyl analysis in citrus fruits. This type of cleanup for the silica gel was apparent-sufficient for the measurement of milligram quantities of diphenyl by a spectrophotometric procedure. BOWYER *et al.*[9] extracted silicic acid with a chloroform–methanol (2:1) mixture to remove lipid contaminants prior to using the silicic acid for the analysis of fatty acids, also in the milligram range. BROWN AND BENJAMIN[10] noted that organic contaminants in commercially available silica gels obscured the desired spots on the acid-sprayed chromatogram and recommended washing the plates with a mixture of diethyl ether–methanol (20:80). AMOS[3] extracted various grades of silica gel with acetone and obtained residues of dark brown oils in amounts ranging from 1.0 to 39 mg per 100 g of gel. KOVACS[11] washed silica gel plates with distilled water prior to use, to remove "chlorides" that would interfere with the $AgNO_3$ spray reagent subsequently used for the detection of pesticides at the 0.10 μg

level. Later, KOVACS[12] discontinued the use of silica gel for pesticide residue analysis and replaced the gel with the adsorbent aluminum oxide, because of the high levels of "chlorinated" impurities in the silica gel. SMITH AND EICHELBERGER[13] used silica gel, as purchased, for the separation of pesticides extracted from a water sample; the amount of each pesticide applied to the TLC plates was about 0.2 mg. Sections of the developed silica gel plates were eluted for gas chromatography confirmatory analysis. Although the suspected pesticides were "confirmed" by this procedure, other unknown components sensitive to the electron capture detector were noted on the gas chromatogram. GEISS *et al.*[4] noted that silica gel contained organic contaminants, and the problem was aggravated by the use of plastic tubing which also contributed volatile contaminants to the gel when the tubing was used in a suction technique for the removal of silica gel sections from the developed plate for further analysis.

In our experimental studies, five different commercially available silica gels, with and without calcium sulfate binder, were found to be contaminated with organic materials which would confuse the interpretation of the gas chromatographic data. Some of the commercial gels were received in plastic bottles, and some were received in aluminum bottles. The contaminants from the gels packed in the aluminum containers, with plastic caps, were less than the amounts found in the plastic-packed gels, but great enough to cause interpretative problems with the analytical data. Experi-

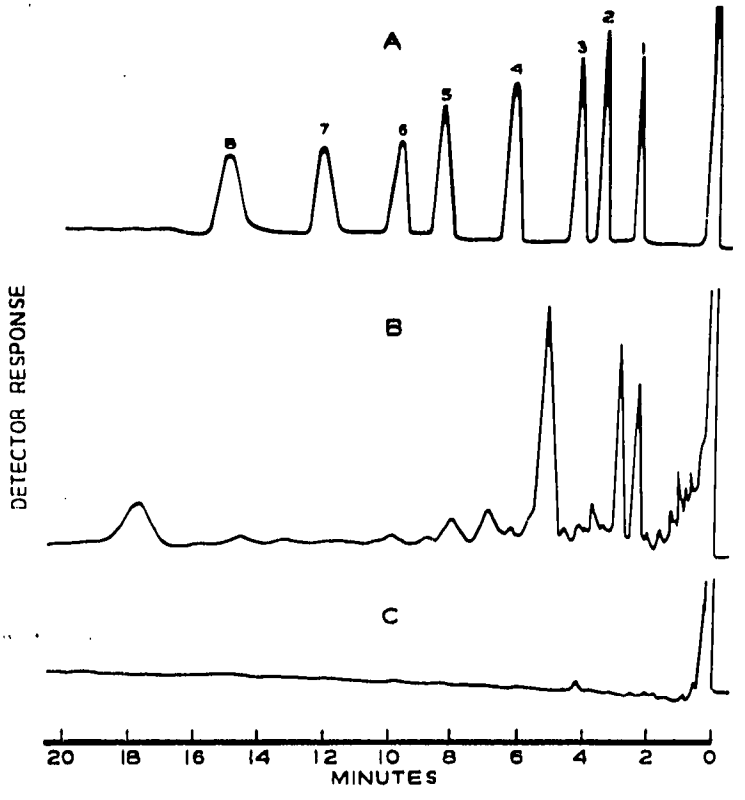

Fig. 1. Gas chromatograph curves obtained from a 1/8 in. × 5 ft. glass column containing 4% SE-30–6% QF-1 silicones on Chromosorb W, HP, 80/100 mesh; column temperature 180°; electron capture detector. (A) Chlorinated pesticide standards: Peaks 1 and 6 are Lindane and Dieldrin, respectively, each 0.3 ng. Peaks 2, 3, 4, 5, 7, 8 are Heptachlor, Aldrin, Heptachlor epoxide, DDE, DDD, DDT, respectively, each 0.6 ng. (B) Contaminants extracted from silica gel prior to heat treatment. (C) Extract of heat-treated silica gel.

ments showed that the plastic containers were at least a partial source of the gel contamination, which confirmed the observations of GEISS *et al.*[4].

Heat treatment of the silica gels at 300° for 16 h effectively removed the contaminants (See Fig. 1); this treatment did not affect the TLC properties of the gels. A less convenient but effective procedure for the removal of contaminants from silica gels is the Soxhlet extraction of the gel with redistilled chloroform for 3 h, followed by extraction with redistilled hexane for 4 h. The Soxhlet cellulose thimbles, if used as extraction containers, must be prewashed in a similar manner.

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